

What is claimed is:

1. An immunogenic composition comprising a mixture of pertactins of *Bordetella* species, wherein said composition comprises:

- (a) pertactin of *Bordetella parapertussis*, and
- (b) pertactin of *Bordetella bronchiseptica*,

in amounts sufficient to induce a humoral or cellular immune response against *Bordetella parapertussis* and *Bordetella bronchiseptica* in an animal to which the immunogenic composition is administered.

2. An immunogenic composition as claimed in claim 1, which also comprises pertactin of *Bordetella pertussis* in an amount sufficient to induce a humoral or cellular immune response against *Bordetella pertussis* in an animal to which the immunogenic composition is administered.

3. An immunogenic composition comprising a mixture of pertactins of *Bordetella* species or fragments thereof, wherein said pertactins or fragments thereof comprise at least two *Bordetella bronchiseptica* pertactin variants and wherein said pertactins of *Bordetella* species or fragments thereof are present in an amount sufficient to induce a humoral or immune response against *Bordetella bronchiseptica* in an animal to which the composition is administered.

4. An immunogenic composition according to claim 3, comprising a mixture of pertactins of *Bordetella* species or fragments thereof, wherein said pertactins or fragments thereof comprise at least two *Bordetella bronchiseptica* pertactin variants, and said at least two *Bordetella bronchiseptica* pertactin variants differ from each other at least in their Region II.

5. An immunogenic composition comprising a mixture of pertactins of *Bordetella* species or fragments thereof, wherein said pertactins or fragments thereof comprise a mixture of *Bordetella bronchiseptica* pertactin variants, wherein each *Bordetella bronchiseptica* pertactin variant comprises 6, 7, 8, or 9 repeating PQP amino acid sequences in Region II thereof, and wherein said *Bordetella bronchiseptica* pertactin variants are present in amounts sufficient to induce a humoral or cellular immune response against *Bordetella bronchiseptica* in an animal to which the immunogenic composition is administered.

6. An immunogenic composition according to claim 5, wherein at least two of the *Bordetella bronchiseptica* pertactin variants differ from each other at least in the number of repeating PQP amino acid sequences in their Region II.

7. An immunogenic composition as claimed in claim 5, which also comprises pertactins of *Bordetella parapertussis* or fragment thereof, *Bordetella pertussis* or fragment thereof, or mixtures thereof, in amounts sufficient to induce a humoral or cellular immune response against *Bordetella parapertussis* or *Bordetella pertussis* in an animal to which the immunogenic composition is administered.

8. An immunogenic composition according to claim 3, comprising a mixture of pertactins of *Bordetella* species or fragments thereof, wherein said pertactins or fragments thereof comprise at least two *Bordetella bronchiseptica* pertactin variants, and said at least two *Bordetella bronchiseptica* pertactin variants differ from each other at least in their Region I.

9. An immunogenic composition comprising a mixture of pertactins of *Bordetella* species or fragments thereof, wherein said pertactins or fragments thereof comprise a mixture of *Bordetella bronchiseptica* pertactin variants, wherein each *Bordetella bronchiseptica* pertactin variant comprises 1, 2, or 3 repeating GGXXP amino acid sequences in Region I thereof, and wherein said *Bordetella bronchiseptica* pertactin variants are present in amounts sufficient to induce a humoral or cellular immune response against *Bordetella bronchiseptica* in an animal to which the immunogenic composition is administered.

10. An immunogenic composition according to claim 9, wherein at least two of the *Bordetella bronchiseptica* pertactin variants differ at least from the number of repeating GGXXP amino acid sequences in their Region I.

11. An immunogenic composition as claimed in claim 9, which also comprises pertactins of *Bordetella parapertussis* or a fragment thereof, *Bordetella pertussis* or a fragment thereof, or mixtures thereof, in amounts sufficient to induce a humoral or cellular immune response against *Bordetella parapertussis* or *Bordetella pertussis* in an animal to which the immunogenic composition is administered.

12. An immunogenic composition comprising a mixture of pertactins of *Bordetella bronchiseptica* species or fragments thereof, wherein said pertactins or fragments thereof comprise a mixture of *Bordetella bronchiseptica* pertactin variants, wherein at least one of said *Bordetella bronchiseptica* pertactin variants comprises Region II of pertactin of *Bordetella bronchiseptica* having 6, 7, 8, or 9 repeating PQP amino acid sequences in Region II thereof, and at least another of said *Bordetella bronchiseptica* pertactin variants comprises Region I of pertactin of *Bordetella bronchiseptica* having 1, 2, or 3 repeating GGXXP amino acid sequences in Region I thereof.

13. An immunogenic composition comprising a mixture of fragments of pertactins of *Bordetella* species, wherein said composition comprises:

- (a) pertactin of *Bordetella parapertussis* or a fragment thereof, and
- (b) pertactin of *Bordetella bronchiseptica* or a fragment thereof containing
Region I, Region II, or both Region I and Region II,

in amounts sufficient to induce a humoral or cellular immune response against *Bordetella parapertussis* and *Bordetella bronchiseptica* in an animal to which the immunogenic composition is administered.

14. An immunogenic composition as claimed in claim 13, which also comprises pertactin of *Bordetella pertussis* or a fragment thereof in an amount sufficient to induce a humoral or cellular immune response against *Bordetella pertussis* in an animal to which the composition is administered.

15. A composition comprising a mixture of at least two *Bordetella bronchiseptica* pertactin variants, wherein each variant comprises Region II of a pertactin of *Bordetella bronchiseptica*, and wherein said variants differ from each other at least in the Region II they each comprise.

16. A composition comprising a mixture of *Bordetella bronchiseptica* pertactin variants, wherein each *Bordetella bronchiseptica* pertactin variant comprises 6, 7, 8, or 9 repeating PQP amino acid sequences in Region II thereof, and at least two *Bordetella bronchiseptica* pertactin variants differ in the number of said repeating PQP amino acid sequences contained therein.

17. A composition as claimed in claim 16, which also comprises pertactins of *Bordetella parapertussis* or a fragment thereof, *Bordetella pertussis* or a fragment thereof, or mixtures thereof.

18. A composition comprising a mixture of at least two *Bordetella bronchiseptica* pertactin variants, wherein each *Bordetella bronchiseptica* pertactin variant comprises Region I of pertactin of *Bordetella bronchiseptica*, and wherein said variants differ from each other at least in the Region I that each comprises.

19. A composition comprising a mixture of *Bordetella bronchiseptica* pertactin variants, wherein each *Bordetella bronchiseptica* pertactin variant comprises 1, 2, or 3 repeating GGXXP amino acid sequences in Region I thereof, and at least two *Bordetella bronchiseptica* pertactin variants differ in the number of said repeating GGXXP amino acid sequences contained therein.

20. A composition as claimed in claim 19, which also comprises pertactins of *Bordetella parapertussis* or a fragment thereof, *Bordetella pertussis* or a fragment thereof, or mixtures thereof.

21. A composition comprising a mixture of *Bordetella bronchiseptica* pertactin variants, wherein one of said *Bordetella bronchiseptica* pertactin variants comprises Region II of pertactin of *Bordetella bronchiseptica* and another of said *Bordetella bronchiseptica* pertactin variants comprises Region I of pertactin of *Bordetella bronchiseptica*.

22. The composition according to claim 21, wherein said Region II has 6, 7, 8, or 9 repeating PQP amino acid sequences.

23. The composition according to claim 21, wherein said Region I has 1, 2, or 3 repeating GGXXP amino acid sequences.

24. A composition comprising a mixture of *Bordetella bronchiseptica* pertactin variants, wherein one of said *Bordetella bronchiseptica* pertactin variants comprises Region II of pertactin of *Bordetella bronchiseptica* having 6, 7, 8, or 9 repeating PQP amino acid sequences in Region II thereof, and another of said *Bordetella bronchiseptica* pertactin variants comprises Region I of pertactin of *Bordetella bronchiseptica* having 1, 2, or 3 repeating GGXXP amino acid sequences in Region I thereof.

25. A composition as claimed in claim 24, which also comprises pertactins of *Bordetella parapertussis* or a fragment thereof, *Bordetella pertussis* or a fragment thereof, or mixtures thereof.

26. A polypeptide comprising a sequence or a fragment of said sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, or SEQ ID NO:22.

27. A polypeptide consisting of the amino acids in SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, or SEQ ID NO:22.

28. A polynucleotide encoding a polypeptide as claimed in claim 26.

29. A purified DNA or RNA sequence that hybridizes under moderate or high stringency conditions to the polynucleotide of claim 28 or at least to 15 nucleotides thereof.

30. A polynucleotide encoding a polypeptide as claimed in claim 27.

31. A purified DNA or RNA sequence that hybridizes under moderate or high stringency conditions to the polynucleotide of claim 30 or at least to 15 nucleotides thereof.

32. Purified antibodies that bind to a polypeptide of claim 26.

33. Purified antibodies according to claim 32, wherein the antibodies are monoclonal antibodies.

34. Purified antibodies according to claim 32, wherein the antibodies are polyclonal antibodies.

35. An immunological complex comprising a polypeptide of claim 26 and an antibody that specifically recognizes said polypeptide.

36. A method for detecting infection by Bordetella, wherein the method comprises providing a composition comprising a biological material suspected of being infected with Bordetella, and assaying for the presence of a polypeptide of claim 26.

37. The method as claimed in claim 36, wherein the polypeptide is assayed by electrophoresis or by immunoassay with antibodies that are immunologically reactive with the polypeptide.

38. An *in vitro* diagnostic method for the detection of the presence or absence of antibodies, which bind to an antigen comprising a polypeptide of claim 26, wherein the method comprises contacting the antigen with a biological fluid for a time and under conditions sufficient for the antigen and antibodies in the biological fluid to form an antigen-antibody complex, and detecting the formation of the complex.

39. The method as claimed in claim 38, which further comprises measuring the formation of the antigen-antibody complex.

40. The method as claimed in claim 38, wherein the formation of antigen-antibody complex is detected by immunoassay based on Western blot technique, ELISA, indirect immunofluorescence assay, or immunoprecipitation assay.

41. A diagnostic kit for the detection of the presence or absence of antibodies, which bind a polypeptide of claim 26 or mixtures thereof, wherein the kit comprises an antigen comprising polypeptide of claim 26 or mixtures of said polypeptides, and means for detecting the formation of immune complex between the antigen and antibodies, wherein the means are present in an amount sufficient to perform said detection.

42. An immunogenic composition comprising at least one polypeptide of claim 26 in an amount sufficient to induce an immunogenic or protective response *in vivo*, and a pharmaceutically acceptable carrier therefor.

43. The immunogenic composition as claimed in claims 1 to 14, wherein said composition comprises a neutralizing amount of at least one polypeptide of claim 26.

44. An immunogenic composition comprising a polynucleotide according to any one of claims 28 to 31.

45. A vaccine comprising an immunogenic composition according to any one of claims 1 to 14, 42, 43 or 44 along with a pharmaceutically acceptable vehicle.

46. A method for detecting the presence or absence of *Bordetella* comprising:

- (1) contacting a sample suspected of containing genetic material of *Bordetella* with at least one nucleotide probe, and
- (2) detecting hybridization between the nucleotide probe and the genetic material in the sample,

wherein said nucleotide probe is complementary to a polynucleotide sequence as claimed in any one of claims 28 or 30.

47. A vaccination kit comprising at least an immunogenic composition according to claims 1 to 14 or 42 to 44, and means for administering the composition to an animal.

48. An immunogenic composition consisting essentially of:

- (A) a polypeptide comprising Region I and Region II, or one polypeptide comprising Region I and one polypeptide comprising Region II, of a pertactin of *Bordetella pertussis*;
- (B) a polypeptide comprising Region I and Region II, or one polypeptide comprising Region I and one polypeptide comprising Region II, of a pertactin of *Bordetella parapertussis*;
- (C) a polypeptide comprising Region I and Region II, or one polypeptide comprising Region I and one polypeptide comprising Region II, of a pertactin of *Bordetella bronchiseptica* strain 9.73 and a polypeptide

comprising Region I and Region II, or one polypeptide comprising Region I and one polypeptide comprising Region II, of a pertactin of *Bordetella bronchiseptica* of strain SEI.

49. An immunogenic composition consisting essentially of:

- (A) a pertactin of *Bordetella bronchiseptica*;
- (B) FHA of *Bordetella bronchiseptica*; and
- (C) a pertactin of *Bordetella parapertussis*.

50. The immunogenic composition as claimed in claim 49, wherein the pertactin of *Bordetella bronchiseptica* is from strain 9.73.

51. The immunogenic composition as claimed in claim 49, wherein the FHA of *Bordetella bronchiseptica* is from strain 9.73.

52. An immunogenic composition as claimed in any one of claims 1 to 14, 42 to 44, or 48 to 51, wherein the composition further comprises at least one adhesin of *Bordetella* selected from the group consisting of FHA, AGG2, AGG3, and/or at least one toxin of *Bordetella* selected from the group consisting of PTX, DNT, TCT, Ac-Hly.

53. A DNA chip, wherein said chip comprises at least one polynucleotide according to claims 28 to 31 or fragment thereof.

54. Use of monoclonal antibodies according to claim 33 for treating *Bordetella* infections.

55. A microarray comprising microbeads, wherein said microbeads each bears multiple copies of a polynucleotide according to any one of claims 28 to 31 or a fragment thereof, and wherein the polynucleotide or fragment thereof is different from one microbead to another.